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# Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms *Fragilariopsis kerguelensis* and *Chaetoceros dicaeta*

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**Abstract.** The interaction between iron availability and the phytoplankton elemental composition was investigated during the in situ iron fertilization experiment EIFEX and in laboratory experiments with the Southern Ocean diatom species *Fragilariopsis kerguelensis* and *Chaetoceros dicaeta*. Contrary to other in situ iron fertilization experiments we observed an increase in the BSi:POC, BSi:PON, and BSi:POP ratios within the iron fertilized patch during EIFEX. This is possibly caused by a relatively stronger increase in diatom abundance compared to other phytoplankton groups and does not necessarily represent the amount of silicification of single diatom cells. In laboratory experiments with *F. kerguelensis* and *C. dicaeta* no changes in the POC:PON, PON:POP, and POC:POP ratios were found with changing iron availability in both species. BSi:POC, BSi:PON, and BSi:POP ratios were significantly lower in the high iron treatments compared to the controls. In *F. kerguelensis* this was caused by a decrease in cellular BSi concentrations and therefore possibly less silicification. In *C. dicaeta* no change in cellular BSi concentration was found. Here lower BSi:POC, BSi:PON, and BSi:POP ratios were caused by an increase in cellular C, N, and P under high iron conditions. These results indicate that iron limitation does not always increase silicification in diatoms and that changes in the BSi:POC, BSi:PON, and BSi:POP ratios under iron fertilization in the field are caused by a variety of different mechanisms. Our results therefore imply that simple cause-and-effect relationships are not always applicable for modeling of elemental ratios.

## 1 Introduction

Recent studies showed that the canonical Redfield ratio of 106:16:1 for C:N:P is not a general stoichiometric optimum for all marine phytoplankton species but rather represents an average of species specific ratios, which can differ extremely from the Redfield ratio (Ho et al., 2004; Klausmeier et al., 2004; Quigg et al., 2003; Twining et al., 2004). It is reported that while the POC:PON ratio is often close to the Redfield ratio, diatoms in general have lower than the Redfield PON:POP and POC:POP ratios (Ho et al., 2004; Quigg et al., 2003). This is supported by observations from the Southern Ocean (SO) where waters dominated by diatoms have lower PON:POP and POC:POP ratios compared to waters dominated by the haptophyte *Phaeocystis antarctica* (Arrigo et al., 2002; Arrigo et al., 1999).

Beside these differences in the elemental composition of different phytoplankton classes, nutrient availability can influence stoichiometry of individual species (see review in Geider and La Roche, 2002). As a possible explanation for deviations from the Redfield ratio the trace metal iron is discussed. Iron is needed in the nitrogen metabolism of phytoplankton cells. For the synthesis of amino acids nitrate has to be reduced to ammonium. This occurs in a two step reduction, where the energy is derived from Fe-dependent photosynthetic redox reactions. Both enzymes involved, nitrate and nitrite reductase, have a high iron content. Additionally nitrite reductase uses reduced ferredoxin, an iron-sulfur redox protein, or the non-iron-containing flavodoxin to reduce nitrite to ammonium. Therefore, iron limitation leads to reduced nitrate uptake rates (Price et al., 1994) and lowers nitrate reductase activity (Timmermans et al., 1994). However, it is not known if the latter is due to a direct reduction in the enzyme activity or indirectly via a reduced supply of the reductant from photosynthesis. Besides nitrate, phytoplankton cells can directly take up ammonium and incorporate

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it into amino acids without the use of iron containing enzymes. Therefore, the iron demand of phytoplankton cells is higher when growing on nitrate compared to ammonium as N source (Maldonado and Price, 1996; Raven, 1988). This implies that in low Fe waters like the SO ammonium uptake is preferred and new production is suppressed (Maldonado and Price, 1996) despite the surplus of nitrate. To fulfill the higher iron requirements for nitrate uptake, phytoplankton cells have higher iron uptake rates when growing on nitrate compared to ammonium (Wang and Dei, 2001).

Therefore, in High Nutrient Low Chlorophyll (HNLC) regions like the SO, where iron limits phytoplankton growth, higher POC:PON and lower PON:POP ratios compared to the Redfield ratio may be expected. However, as many other abiotic factors such as macronutrient concentration, daylength, irradiance, salinity, and temperature also have a wide influence on the elemental stoichiometry the overall situation is more complicated (Geider and La Roche, 2002). As the POC:PON ratio is rather constant independent of phytoplankton species (Ho et al., 2004; Quigg et al., 2003) and iron concentration (Greene et al., 1991; Price, 2005), while the POC:POP and PON:POP ratios are much more variable, intracellular phosphate seems to be more influenced by iron availability. The exact mechanisms how the elemental composition of phytoplankton is influenced by iron and why there are large species specific differences remain unknown.

Besides nitrate uptake, iron affects the efficiency of the photosynthetic apparatus and thus probably carbon uptake. Iron is an essential part of the iron-sulfur proteins and ferredoxin of the photosystems and the heme and iron-sulfur proteins of the cytochrome  $b_6f$  complex. It therefore plays an important role in the photosynthetic electron transfer and is essential for photosynthetic energy supply (Greene et al., 1991; Greene et al., 1992; Greene et al., 1994). Under iron limitation a visible decrease in chlorophyll concentration (chlorosis) as well as a decrease in the photosynthetic efficiency ( $F_v/F_m$ ) is generally observed.

Besides the impact on POC, PON, and POP composition, one important effect of iron limitation on the elemental stoichiometry is an increase in the BSi:POC, BSi:PON, and BSi:POP ratio of diatoms (Hutchins and Bruland, 1998; Price, 2005; Takeda, 1998; Timmermans et al., 2004; Twinning et al., 2004). While this can be caused by decreased cellular Si levels upon release from iron stress (Hutchins and Bruland, 1998; Takeda, 1998) other studies show the effect to be driven mainly by increase in cellular nitrogen and carbon with little or no change in cellular Si (Franck et al., 2003; Takeda, 1998). It is generally assumed that higher silicification is caused by a reduction in growth rate and an increased duration of the cell in the  $G_2 + M$  phase of the cell cycle during which Si uptake occurs (Martin-Jézéquel et al., 2000). Therefore, the effect of iron on the BSi:POC, BSi:PON, and BSi:POP ratios is an indirect one and the same effect is observed for other factors influencing growth such as temperature, light intensity (Brzezinski, 1985), photoper-

iod, and macronutrient limitation (reviewed by Ragueneau et al., 2000). However, data on the cellular elemental composition of SO diatoms under different iron availabilities are rare and it is therefore not certain that iron fertilization generally decreases frustule silicification. The resulting effects on grazing protection, sinking rates, and remineralization have to be considered in relation to the species specific response to iron availability.

In this study we examined the effect of iron deplete and replete growth conditions on the elemental composition of the two Antarctic diatom species *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*. These species were selected because of their important contribution to the diatom biomass in the SO community. Further, they represent two different degrees of silicification with *F. kerguelensis* being stronger silicified compared to *C. dichaeta*. These results were compared to size fractionated BSi:POC, BSi:PON, and BSi:POP ratios during the in situ iron fertilization experiment EIFEX.

Aim of this study is to investigate the effect of iron on silicification of two important SO diatom species and thus to help interpreting changes in the BSi:POC, BSi:PON, and BSi:POP ratios observed in field experiments.

## 2 Material and methods

A detailed description of the phytoplankton community structure, total and size fractionated POC, PON, POP, as well as total BSi concentrations and corresponding molar ratios during EIFEX is given by Hoffmann et al. (2006). Here we present new information on the POC, PON, POP, and BSi concentrations as well as the BSi:POC, BSi:PON, and the BSi:POP molar ratios in the size fractions  $>20 \mu\text{m}$  and the  $<20 \mu\text{m}$  during EIFEX together with results from culture experiments with the SO diatom species *F. kerguelensis* and *C. dichaeta*.

*F. kerguelensis* and *C. dichaeta* were isolated on board RV "Polarstern" during the SO iron fertilization experiment EIFEX. Single cells were isolated under a light microscope using small glass pipettes and were rinsed at least three times in sterile filtered Antarctic seawater.

The species were grown under iron limitation in the IfM-GEOMAR culture collection at  $3^\circ\text{C}$ . Special care was taken to prevent contamination with iron. Every procedure was done under trace metal clean conditions in a laminar flow bench. All materials coming into contact with the cultures and/or the medium were HCl rinsed before use. Sterile filtered Antarctic seawater enriched with macronutrients, vitamins, and EDTA buffered trace metals (except for iron), all in  $f/2$  concentrations, was used as culture medium. The light climate was  $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  provided by cool fluorescence tubes (OSRAM FLUORA L18 W/77 and BIOLUX 18 W/965) at a 16 h:8 h light:dark cycle.

The culture media for all experimental treatments was prepared as described above except for iron concentrations.

**Table 1.** Iron and EDTA concentrations in the different treatments of the two laboratory experiments.

Species	Treatment			
	A	B	C	D
<i>F. kerguelensis</i>	no Fe addition no EDTA	no Fe addition 10 $\mu$ M EDTA	1.55 nM Fe' 10 $\mu$ M EDTA	15.5 nM Fe' 10 $\mu$ M EDTA
<i>C. dictyota</i>	–	no Fe addition 10 $\mu$ M EDTA	1.55 nM Fe' 10 $\mu$ M EDTA	15.5 nM Fe' 10 $\mu$ M EDTA

Handling during the experiment was again done under trace metal clean conditions as described above

Two experiments with three iron treatments and EDTA were carried out: one treatment with EDTA only, one treatment with EDTA and 100 nM Fe addition, and one treatment with EDTA and 1000 nM Fe added. Three replicates were incubated for each treatment.

In the two iron enrichment treatments, respective free iron concentrations (Fe', including all inorganic iron species) were 1.55 nM Fe' and 15.5 nM Fe' as estimated after Timmermans et al. (2001). An additional experiment was carried out with *F. kerguelensis* without iron and EDTA addition in order to investigate the effect of EDTA on growth and stoichiometry. Culture conditions and treatment labels are listed in Table 1.

Samples for chlorophyll measurements were filtered on GF/F filters (Whatman) and immediately stored at  $-20^{\circ}\text{C}$  until analysis. The frozen filters were put in polypropylene vials and 11 ml 90 % acetone and glass beads (2 mm and 4 mm) were added. Thereafter, the closed vials were put in a cell mill for at least 5 min until the filters were completely homogenized. The vials were then centrifuged at  $-5^{\circ}\text{C}$  (10 min at 5000 rpm). The extract was carefully taken by a pipette and filled in 5 cm glass cuvettes. Extinction was measured photometrically based on Jeffrey and Humphrey (1975).

The photosynthetic efficiency (Fv/Fm) was measured using a PhytoPAM (Walz, Germany) based on Kolbowski and Schreiber (1995). Samples were dark adapted for 10 min and kept on ice directly before measurement.

For determination of cell numbers 2 ml samples were fixed with 40  $\mu$ l Lugol's Solution (iodine – potassium iodide solution 1 %, MERCK) and stored at  $3^{\circ}\text{C}$  in the dark until analysis. Cells counts were performed using light microscopy (Utermöhl and Axiovert 100) at different magnifications according to the size of the organisms. In each sample at least 500 cells or the total volume was counted.

Size fractionated samples for POC, PON, POP, and BSi measurements during EIFEX were taken and treated as described by Hoffmann et al. (2006). POC, PON, POP, and BSi samples from culture experiments were not size fractionated.

POC and PON measurements of the EIFEX and *F. kerguelensis* samples were analyzed using a C/N Analyser (Euro

EA Elemental Analyser) after Ehrhard and Koeve (1999). The POP content of all samples was determined colorimetrically using the method from Hansen and Koroleff (1999). BSi was transformed to dissolved silicate by heating the filters in 0.1 mol L $^{-1}$  NaOH solution to  $85^{\circ}\text{C}$  for 2 h. The dissolved silicate was then determined colorimetrically (Hansen and Koroleff, 1999). HPLC pigments measurements were carried out as described by Hoffmann et al. (2006) using a method modified after Barlow et al. (1997). The POC and PON content of the *C. dictyota* cultures was analyzed using an Euro EA-CN IRMS elemental analyzer linked to Finnegan Delta Plus radio isotope mass spectrometer as described by Carman and Fry (2002). Acetanelid and peptone were used as standards.

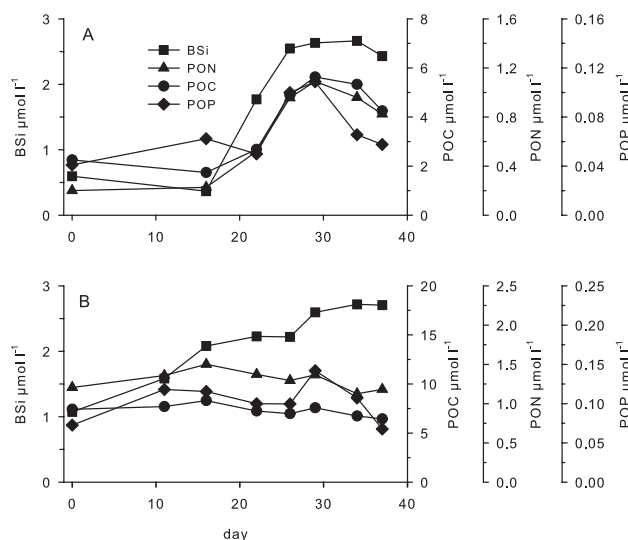
Growth rates of *F. kerguelensis* and *C. dictyota* in culture experiments were calculated as  $\mu = (t_2 - t_1)^{-1} \times \ln(N_2/N_1)$  where  $\mu$  is the net growth rate  $d^{-1}$  and  $N_1$  and  $N_2$  are the cell concentrations at  $t_1$  and  $t_2$ , respectively. For statistical analysis Students t-test was used. Differences found are reported as significant in the text if  $p < 0.05$ .

### 3 Results

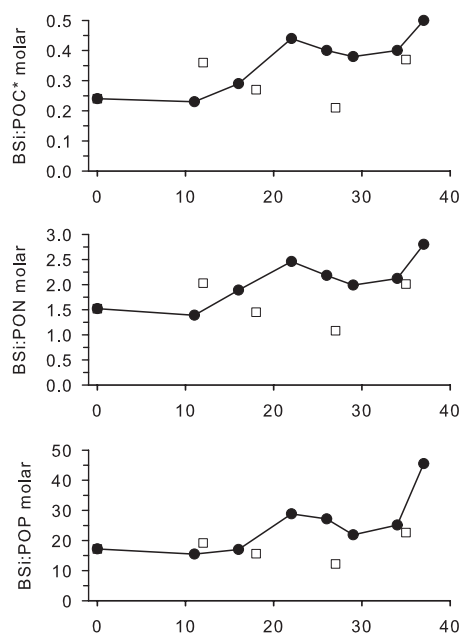
#### 3.1 Size fractionated particulate organic matter during EIFEX

During EIFEX, the composition of particulate organic matter (POM) inside the iron fertilized patch showed different temporal trends in the  $>20 \mu\text{m}$  and the  $<20 \mu\text{m}$  size fractions (Fig. 1). Almost no changes were found in the  $>20 \mu\text{m}$  size fraction during the first 16 days of the experiment. After day 16, BSi, POC, PON, and POP concentrations increased by a factor of 4.1, 1.9, 4.1, and 1.4, respectively (Fig. 1a). In the size fraction  $<20 \mu\text{m}$ , no changes in POC, PON, and POP concentrations were observed but BSi concentrations increased continuously until the end of the experiment, on day 37 after the first fertilization. The final BSi concentration in the size fraction  $<20 \mu\text{m}$  was 2.5 times the initial value at the end of the experiment (Fig. 1b).

Inside the fertilized patch, the BSi:POC, BSi:PON, and BSi:POP ratios of the total biomass increased until the end of the experiment. Final values were higher by a factor

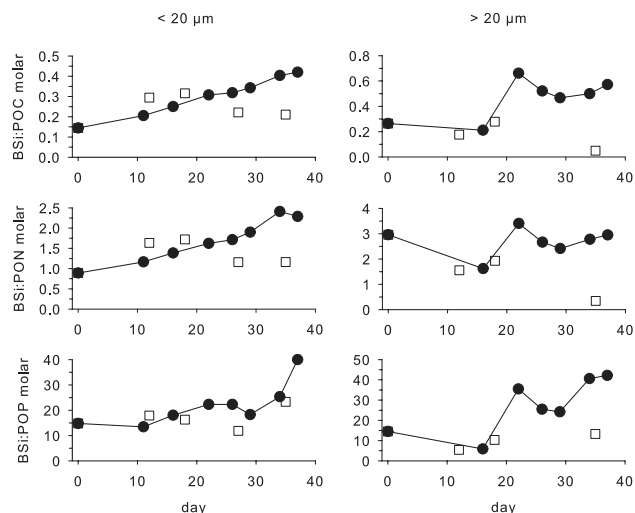


**Fig. 1.** Concentrations of BSi, POC, PON, and POP in the  $>20\ \mu\text{m}$  size fraction (A) and in the  $<20\ \mu\text{m}$  size fraction (B) during EIFEX.

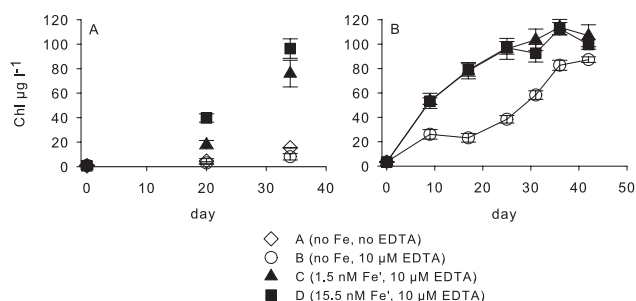


**Fig. 2.** Molar ratios of BSi:POC, BSi:PON, and BSi:POP of the total biomass during the iron fertilization experiment EIFEX inside (dark circles) and outside the iron fertilized patch (open squares). \*Hoffmann et al. (2006)

of 2.1, 1.3, and 2.6, respectively. In non-fertilized water the ratios were relatively patchy, however, no trend was observed (Fig. 2). Separation of the total biomass in  $>20\ \mu\text{m}$  and  $<20\ \mu\text{m}$  size fractions shows that the same trends were found in the molar ratios of both size classes (Fig. 3). The BSi:POC, BSi:PON, and BSi:POP ratios increased continuously in the  $<20\ \mu\text{m}$  size fraction from 0.15, 0.9, and 14.8



**Fig. 3.** Molar ratios of the  $<20\ \mu\text{m}$  and the  $>20\ \mu\text{m}$  size fraction inside the iron fertilized patch (dark circles) and outside the iron fertilized patch (open squares) during EIFEX.



**Fig. 4.** Chlorophyll concentrations in cultures of *F. kerguelensis* (A) and *C. dictyota* (B) grown under different iron concentrations.

at the start of the experiment to 0.4, 2.3, and 40.0 on day 37 inside the fertilized patch. Outside the patch the values were lower in the second half of the experiment. In the  $>20\ \mu\text{m}$  fraction the elemental ratios did not increase steadily. The values were 0.26 (BSi:POC), 3.0 (BSi:PON), and 14.5 (BSi:POP) in the beginning and decreased within the first 16 days of the experiment, followed by a large increase at day 21 to 0.7, 3.4, and 35.5. During the rest of the experiment the values stayed at an elevated level.

### 3.2 Growth parameters in the laboratory experiments

In culture experiments with *F. kerguelensis* and *C. dictyota*, iron addition resulted in a significant increase in photosynthetic efficiency ( $F_v/F_m$ ), maximum growth rates, and chlorophyll concentrations as compared to the control treatments (Fig. 4 and Table 2). Growth rates, Chl concentrations, and  $F_v/F_m$  values were not statistically different in both low iron treatments in *F. kerguelensis* (treatments A and B; t-test;  $p=0.3\text{--}0.6$ ). Iron addition resulted in a distinct

**Table 2.** Cellular elemental composition and chlorophyll concentration, growth rate and maximum Fv/Fm of *F. kerguelensis* at day 34 in the two low iron treatments A and B and the high iron treatments C and D and of *C. dictyota* at day 31 in the low iron treatment B and the high iron treatments C and D. Values marked with \* were significantly different (t-test;  $p < 0.05$ ) between high and low iron treatments. Values marked with ° were only significantly different between treatment B and C.

Treatment	$\mu_{\max} \text{ d}^{-1}$	Fv/Fm max	Chl $\text{pg cell}^{-1}$	Elemental composition			
				C $\text{pmol cell}^{-1}$	N $\text{pmol cell}^{-1}$	P $\text{pmol cell}^{-1}$	Si $\text{pmol cell}^{-1}$
<i>F. kerguelensis</i>	*	*	*				*
A	0.12±0.01	0.29±0.08	1.9±0.8	17.4±4.1	2.9±0.5	0.7±0.2	14.2±0.5
B	0.10±0.01	0.29±0.05	2.3±0.9	15.7±1.3	2.5±0.2	0.7±0.1	12.0±1.0
C	0.18±0.0	0.44±0.06	3.6±0.8	21.5±2.6	4.0±0.3	1.0±0.2	9.0±1.2
D	0.20±0.01	0.60±0.05	4.5±0.9	19.3±6.3	3.7±1.3	1.0±0.2	9.1±1.3
<i>C. dictyota</i>	*	*	*	°	°	°	
B	0.22±0.01	0.32±0.00	0.4±0.2	2.0±0.03	0.4±0.0	0.05±0.0	0.6±0.1
C	0.31±0.03	0.54±0.02	1.1±0.2	6.1±0.7	1.1±0.1	0.1±0.03	0.7±0.1
D	0.31±0.02	0.56±0.02	0.8±0.1	3.5±0.7	0.6±0.1	0.08±0.02	0.6±0.2

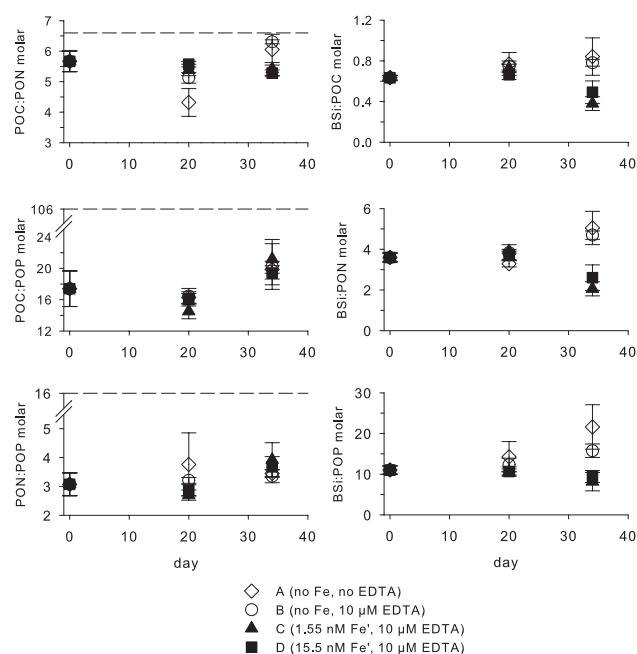
increase in each of these parameters with higher values at 15.5 nM Fe' compared to 1.55 nM Fe'. At the beginning of the experiment, mean chlorophyll concentrations were  $0.8 \mu\text{g l}^{-1}$  for all treatments. The chlorophyll concentrations increased to  $15.4 \mu\text{g l}^{-1}$  in 34 days without iron and EDTA addition (Fig. 4a). Under EDTA addition Chl concentrations were slightly lower ( $8.1 \mu\text{g l}^{-1}$ ). The addition of 1.55 nM Fe' resulted in a significant increase in the chlorophyll concentrations compared to both treatments without iron addition (t-test;  $p=0.003$  and  $0.0007$ ). After 34 days, Chl concentrations reached a value of  $76 \mu\text{g l}^{-1}$ , which is a 95 times increase. Higher iron concentrations of 15.5 nM Fe' additionally increased Chl concentrations to a value of  $96.4 \mu\text{g l}^{-1}$ . At the beginning of the experiment, Fv/Fm values of *F. kerguelensis* were between 0.15–0.29. These low values indicated that the start culture was iron limited before the experiment. However, the variance here and in the low iron treatments throughout the experiment was relatively high, possibly caused by the very low biomass. Without iron addition maximum Fv/Fm values of 0.29 were reached. Under iron addition Fv/Fm values increased to 0.44 (treatment C) and 0.6 (treatment D) (Table 2). Maximum growth rates roughly doubled from 0.10 and 0.12 in the low iron treatments to 0.18 and 0.2 in the high iron treatments (Table 2).

In *C. dictyota* Chl concentrations in both high iron treatments increased rapidly about 30 times to 96.1 and  $97.0 \mu\text{g l}^{-1}$  in 25 days (Fig. 4b). Thereafter, concentrations leveled off and highest values were reached at day 36 with  $113.9 \mu\text{g l}^{-1}$  (treatment C) and  $112.8 \mu\text{g l}^{-1}$  (treatment D). In the low iron treatment Chl concentrations increased only 11.6 times until day 25. However, growth continued throughout the experiment reaching concentrations almost as high as the high iron treatments of  $87.3 \mu\text{g l}^{-1}$  at day 42. At the be-

ginning of the experiment, Fv/Fm values were between 0.19 and 0.21 and increased more than two times reaching maximum values of 0.54 (treatment C) and 0.56 (treatment D) after iron addition (Table 2). Under iron limitation the maximum Fv/Fm value was 0.32 and the maximum growth rate was 0.22 (Table 2). Unlike as in *F. kerguelensis*, we found no difference between the addition of 1.55 and 15.5 nM Fe'. In both high iron treatments the maximum growth rate was 0.31.

### 3.3 Elemental ratios in the laboratory experiments

In both species no significant changes in the POC:PON, POC:POP, and PON:POP ratios were found between the high and low iron treatments and the different growth periods (Figs. 5 and 6). However, all elemental ratios were below the Redfield ratio of C:N:P 106:16:1. *C. dictyota* and *F. kerguelensis* had mean POC:PON ratios of 5.8 and 5.5, which is lower than the Redfield ratio of 6.6. More severe are the differences in the POC:POP and PON:POP ratios which are 44.6 and 7.7 in *C. dictyota* and 17.8 and 3.3 in *F. kerguelensis*, respectively. Here the difference from the Redfield POC:POP ratio of 106 is 58% (*C. dictyota*) and 83% (*F. kerguelensis*). The deviation from the Redfield PON:POP ratio of 16 is 52% (*C. dictyota*) and 80% (*F. kerguelensis*). In both species high iron concentrations resulted in lower BSi:POC, BSi:PON, and BSi:POP ratios compared to the low iron treatments. At day 34 (*F. kerguelensis*) and day 31 (*C. dictyota*) the ratios were roughly twice as high in the low iron treatment compared to the high iron treatments.



**Fig. 5.** Molar elemental ratios in *F. kerguelensis* grown under different iron and EDTA concentrations.

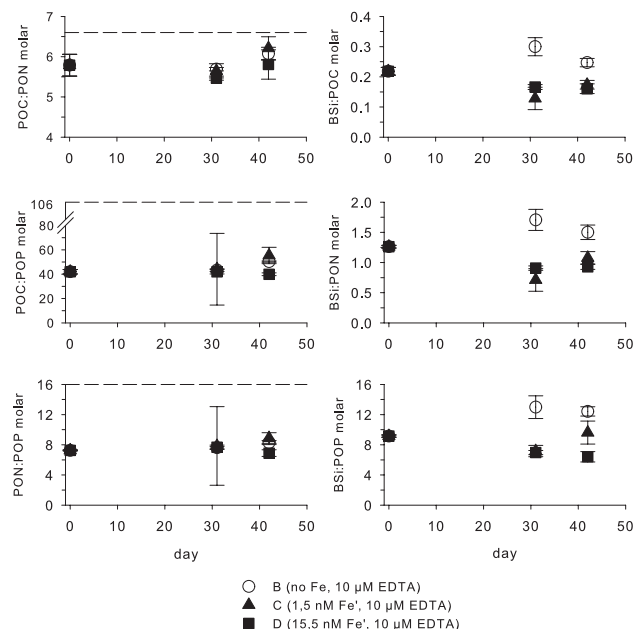
### 3.4 Cellular composition

Chl per cell increased in all high iron treatments in both species. Cellular chlorophyll concentrations of *F. kerguelensis* and *C. dictyota* were about two times higher in the high iron treatments (Table 2). Interestingly, the cellular elemental composition shows different strategies of the two species both resulting in lower BSi:POC, BSi:PON, and BSi:POP ratios under high iron concentrations. Mean cellular C, N, and P concentrations of all treatments were 18.9, 3.6, and 1.0 pmol cell<sup>-1</sup> in *F. kerguelensis* showing no significant changes due to iron concentrations (t-test;  $p=0.1-0.9$ ). However, BSi concentrations per cell were significantly higher in both low iron treatments compared to the high iron treatments (t-test;  $p=0.035$  and  $0.039$ ). In contrast to that no significant change in the cellular BSi concentration in *C. dictyota* was found (mean of 0.7 pmol cell<sup>-1</sup>; t-test;  $p=0.2-0.9$ ), while cellular C, N, and P concentrations were about twice as high under high iron concentrations.

## 4 Discussion

### 4.1 Deviation from the Redfield ratio

The elemental composition of diatoms is known to be extremely variable between different species (Sarhou et al., 2005). A general observation from laboratory experiments (Ho et al., 2004; Price, 2005; Quigg et al., 2003) and field studies (Arrigo et al., 1999; Coale et al., 2004; Hoffmann et



**Fig. 6.** Molar elemental ratios in *C. dictyota* grown under different iron and EDTA concentrations.

al., 2006) is that the mean POC:PON ratio of diatoms is close to the Redfield ratio of 6.6 and shows only minor changes due to environmental conditions. In agreement to that we found mean POC:POP ratios of 5.5 for *F. kerguelensis* and 5.8 for *C. dictyota* that showed no significant changes with iron concentration. The PON:POP and POC:POP ratios of diatoms are generally lower than the Redfield ratio (Fu et al., 2005; Ho et al., 2004; Quigg et al., 2003; Sarhou et al., 2005) and are much more susceptible to changes in nutrient supply. For *Thalassiosira weissflogii* a higher accumulation of P and resulting lower PON:POP and POC:POP ratios are reported under iron limiting conditions in laboratory experiments (Price, 2005). It remains unknown if this is due to a luxury uptake and storage of P or if specific physiological processes under iron limitation force the cell to a higher P usage. However, the elemental PON:POP and POC:POP ratios of both species tested in this study were not affected by the iron concentration of the growth medium (Figs. 5 and 6). Similar findings are reported for the PON:POP ratio in *C. dictyota* and *Nitzschia* sp. (Takeda, 1998) and *Phaeodactylum tricornutum* (Greene et al., 1991). Takeda (1998) showed that in vitro iron fertilization can lead to opposed changes in the ratio of  $\text{NO}_3^-:\text{PO}_4^{3-}$  consumption depending on the oceanic region. While he found an increase in the ratio of  $\text{NO}_3^-:\text{PO}_4^{3-}$  consumption in the SO, no changes were found in the Subarctic North Pacific and a decrease is reported from waters of the Equatorial Pacific. Hoffmann et al. (2006) report a wide variability between the PON:POP and POC:POP ratios of the different phytoplankton size classes after iron fertilization in the SO. While both

ratios increased from far below Redfield values to close to Redfield values in the microplankton, the opposite trend was observed in the nanoplankton. Here, start values close to the Redfield ratio decreased to far lower values after iron fertilization.

Although we found no changes in the relative elemental composition of both species and in the absolute cellular C, N, and P concentrations in *F. kerguelensis*, total cellular C, N, and P concentrations roughly doubled with higher iron concentration in *C. dictyota* (Table 2). This effect may partly be caused by a change in cell volume. We found an increase in the cell volume of *C. dictyota* by a factor of 1.3 with higher iron concentrations (data not shown). Assuming constant cellular elemental composition, this increase in cell volume would also result in a 1.3 times higher cellular C, N, P, and Si concentration. In fact, the cellular C, N, and P concentration increased 3.0, 3.0, and 2.2 times in the high iron treatment C compared to the low iron treatment B and 1.7, 1.8, and 1.8 times in the high iron treatment D compared to treatment B (compare Table 2). Therefore, the observed increase in cell volume under higher iron availability can not explain all of the observed increase in cellular C, N, and P concentrations. Additionally a higher C, N, and P accumulation must have taken place in this species under high iron concentrations. It can be speculated that increased C uptake due to higher photosynthetic activity and higher nitrate uptake under high iron concentrations are responsible for this observation. To our knowledge no physiological effect is known that could explain increased P uptake under high iron concentrations. No change in cell size of *F. kerguelensis* was found during this experiment, which is consistent with findings reported by Timmermans et al. (2004) for the same species. These authors showed that the cellular nutrient consumption ratios of four SO diatom species grown under different iron concentrations differ extremely and showed no collective trend. Cellular N consumption increased in *Actinocyclus* sp., *Thalassiosira* sp., and *C. pennatum* with increasing iron concentration, while no changes were found in *F. kerguelensis*. In agreement with our observations, none of the species tested by Timmermans et al. (2004) showed higher cellular P uptake under iron limitation. The absolute values for *F. kerguelensis* reported by Timmermans et al. (2004) are lower than those presented in this study. To avoid confusion it should be noted in this context that the data reported by Timmermans et al. (2004) are cellular  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  consumption rates estimated by the decrease of these nutrients in the culture medium. The absolute values can therefore not directly be compared to particulate N and P concentrations presented in this manuscript.

These results show that luxury P consumption, as reported by Price (2005) for *T. weissflogii*, is not a general mechanism controlled by iron availability, but rather a species specific reaction. It has been recently shown that phytoplankton cells absorb P on their cell surfaces in an amount of 14% to 57% of total cellular P (Fu et al., 2005; Sañudo-Wilhelmy et al.,

2004). The measured PON:total POP and POC:total POP ratios might therefore generally be lower than the truly intracellular stoichiometry and thus may falsify our interpretation of the nutritional status of the cells. When corrected for the surface bound P Fu et al. (2005) describe an increase in the C:intracellular P and N:intracellular P ratios by a factor of 1.2 to 2 in all species tested, including one Southern Ocean *Chaetoceros* species. It can only be speculated what the effect of these observations on the proposed POP export may be. It is possible that sinking cells would lose a lot of the surface bound P due to microbial uptake. This could possibly decrease the amount of total P exported to the deep sea greatly. These uncertainties lead us to the suggestion to use PON:POP and POC:POP ratios with great caution in terms of nutrient drawdown ratios and for biogeochemical modeling. The general observation that the POC:PON ratio is less affected by environmental conditions and generally closer to the Redfield ratio (Geider and La Roche, 2002) makes it a far better proxy for these purposes.

#### 4.2 Impact of iron on silicification

A collective observation from in situ iron fertilization experiments is that the growth of diatoms, especially large species, is stimulated to a greater degree than other phytoplankton groups. In these experiments, besides the increase in cell counts and chlorophyll concentrations, the drawdown of nitrate in iron fertilized waters is often used to follow the biomass development. As iron is an essential component in the enzymes responsible for nitrogen uptake, nitrate and nitrite reductase, it was not surprising to see that nitrate uptake was greatly enhanced by iron fertilization. More remarkable was the observation that higher iron availability decreased the BSi:PON ratio in bottle incubation experiments in all HNLC regions (Brzezinski et al., 2003; De La Rocha et al., 2000; Franck et al., 2003; Franck et al., 2000; Hutchins and Bruland, 1998; Martin and Fitzwater, 1988; Takeda, 1998; Watson et al., 2000). This phenomenon is not only caused by increasing N uptake but also by lower cellular BSi concentrations (Hutchins and Bruland, 1998; Takeda, 1998) and thus also affected the BSi:POC and BSi:POP ratios. Because of the relative increase in BSi under iron limitation, Boyle (1998) suggested that iron-limited diatoms grow thicker and thus heavier silica shells, which sink faster to the sea floor and are less remineralized. This was thought to be a reason for the high accumulation rates of diatom frustules in SO sediments despite relatively low BSi production in the euphotic zone, known as the “opal paradox” (Nelson et al., 1995; Tréguer et al., 1995). It is discussed that the “opal paradox” may result from an underestimation of BSi production and an overestimation of BSi burial rates in the sediments (Pondaven et al., 2000). However, more recent research supports the exceptionally high BSi accumulation rates in SO sediments (Rickert et al., 2002).



Contrary to other in situ iron fertilization experiments, which showed decreasing BSi:PON and BSi:POC ratios, respectively (Boyd et al., 2005; Coale et al., 2004; Gall et al., 2001), the BSi:POC, BSi:PON, and BSi:POP ratios of the total biomass increased 1.8 to 2.6 times during EIFEX (Fig. 2). This is caused by a stronger increase of BSi concentrations compared to POC, PON, and POP concentrations, which can be explained by a relative increase in the diatom abundance and a shift towards diatom species that are stronger silicified compared to others (Hoffmann et al., 2006).

In the  $>20\ \mu\text{m}$  size fraction, concentrations of BSi, POC, PON, and POP increased inside the fertilized patch (Fig. 1a). Here again increasing BSi:POC, BSi:PON, and BSi:POP ratios result from a stronger enhancement in BSi concentrations compared to the other parameters. As this size fraction was dominated by diatoms from the beginning (Hoffmann et al., 2006), this observation can only be caused by a shift in the diatom community structure towards heavily silicified species. In the  $<20\ \mu\text{m}$  size fraction no increase in POC, PON, and POP concentrations was found, while BSi concentrations increased steadily throughout the experiment (Fig. 1b). In this size fraction cell counts and HPLC pigment data show a shift from a haptophyte dominated towards a diatom dominated phytoplankton community (Hoffmann et al., 2006). This resulted in increasing BSi concentrations, while POC related biomass showed no changes. Changes in species composition during EIFEX may have been more pronounced compared to other in situ iron fertilization experiments. This shift in the diatom assemblage towards more heavily silicified species may have overwhelmed any reduction in the Si content of individual cells.

To further understand these mechanisms and explain the differences between EIFEX and other experiments, we performed laboratory experiments with the two SO diatoms *F. kerguelensis* and *C. dictyota*, which were both important species during EIFEX, and determined cellular POC, POP, PON, and BSi concentrations. The BSi:POC and BSi:PON ratios of *F. kerguelensis* were relatively close to those found in the  $>20\ \mu\text{m}$  size fraction and in the total community towards the end of the experiment in the field (Figs. 2, 3 and 5). This observation is not surprising since *F. kerguelensis* has been the most abundant species during EIFEX (Assmy et al., 2005). However, the BSi:POP ratios were lower in both cultures. As described above the cellular P pool is more complex than the C and N pools due to the existence of surface bound P. The surface bound P concentration is dependent on the amount of surface bound Mn (Sañudo-Wilhelmy et al., 2004), which is explained by the adsorption of P to cell-surface-bound Mn hydroxides and oxides. In the SO Mn concentrations are known to be very low (Martin et al., 1990). It is therefore possible that in this region surface bound P concentrations and thus total cellular P concentrations are lower compared to our laboratory experiments, where all trace metals, including Mn, were added in excess. This could possibly explain the higher BSi:POP ratios observed in the field

during EIFEX. This assumption is further supported by the observation that POC:POP and PON:POP ratios of all size classes during EIFEX reported by Hoffmann et al. (2006) were higher compared to those found in *F. kerguelensis* and *C. dictyota* in this study (Figs. 5 and 6).

Our laboratory experiments show that increased cellular BSi concentrations under iron limitation are not a general phenomenon of all diatom species. In both species tested we observed higher BSi:POC, BSi:PON, and BSi:POP ratios under low iron availability. However, while BSi concentrations per cell were significantly higher under iron limitation in *F. kerguelensis*, no changes were found in *C. dictyota* (Table 2). Even though cell volume was lower by a factor of 1.3 under iron limitation in the latter species Si concentrations per cell volume showed no significant difference as well ( $0.44\pm0.07$  in the low iron treatment B and  $0.4\pm0.06$  and  $0.32\pm0.11$  in the high iron treatments C and D). In this species the increases in the BSi:POC, BSi:PON, and BSi:POP ratios are caused by lower cellular C, N, and P concentrations as described above. Analysis of Si consumption per cell led to similar results showing higher cellular Si accumulation under low iron concentrations in *Actinocyclus* sp., *Thalassiosira* sp., and *F. kerguelensis*, but no significant change in cellular Si accumulation in *Corethron pennatum* (Timmermans et al., 2004). We suggest that species specific changes in the elemental composition as well as the reactions of other phytoplankton groups and the heterotrophic biomass conceal the effect of iron on diatom silicate uptake in the field. Additionally, internal Si pools can provide a large amount of cellular Si (see below) (Martin-Jézéquel et al., 2000). Changes in the BSi:POC, BSi:PON, and BSi:POP ratios with iron fertilization should thus be interpreted carefully in terms of diatom silicification.

The reason for changes in nutrient uptake and storage under changing iron availability is, with the exception of nitrate, not well understood. Fe limitation directly decreases the uptake rates of SO diatoms for silicic acid (Brzezinski et al., 2005; De La Rocha et al., 2000; Franck et al., 2003; Franck et al., 2000). However, it has been hypothesized that increased Si uptake is caused by an increased duration of the cell wall synthesis phase. Si uptake is closely related to the  $G_2$ +M phase of the cell cycle. Nutrient (N, Fe), light, or temperature limitation that prolong this phase lead to higher silicification in diatoms (see review in Martin-Jézéquel et al., 2000). Despite lower uptake rates under Fe limitation the increased period available for Si uptake resulted in higher silicification of diatom frustules. However, our results and those of previous studies show that this phenomenon is not valid for all diatom species. The observed changes in the cellular nutrient concentrations resulted in the same changes in the BSi:POC, BSi:PON, and BSi:POP ratios for both species. Therefore, these changes in the cellular concentrations will be of less importance for analysis of nutrient budgets. However, they can possibly affect the sinking behavior as well as the remineralization of frustules in the sediments. One of

the common goals of in situ iron fertilization experiments is to evaluate the effect of iron limitation on carbon export to the deep sea. Therefore, the impact of iron on sedimentation and remineralization is of great interest. Iron can influence the sinking rates of diatoms in two ways: via an impact on the cell buoyancy or via an impact on frustule silicification and thus dry weight. Usually diatom cells control buoyancy by the selective exchange of heavier and lighter ions in the vacuole, the so-called ionic pump, which requires a lot of energy (Anderson and Sweeney, 1978). Iron limitation might reduce the efficiency of energy-producing pathways needed by the cells to maintain buoyancy (Sarhou et al., 2005). However, diatoms are known to dominate the phytoplankton community in turbulent waters (Harris, 1986). It is therefore not likely that a successful phytoplankton group such as diatoms suffers from permanent stress to maintain cell buoyancy in regions like the SO, which are characterized by low iron concentrations and extremely deep mixing throughout the year. Higher sinking rates were observed for iron limited cells of *Actinocyclus* sp. (Muggli et al., 1996) and of *F. kerguelensis*, *Nitzschia* sp., and *Navicula* sp. during the *in situ* iron fertilization experiment SOIREE (Waite and Nodder, 2001). This phenomenon is thought to be a mechanism for the preservation of a seed population or could possibly give the cells access to higher nutrient concentrations in deeper waters (De La Rocha et al., 2000). The largest diatom cells during SOIREE, *Trichotoxon* sp. and *Thalassiothrix* sp., showed only little changes in sinking rates (Waite and Nodder, 2001). The role of silicification in diatom sinking rates is discussed controversially and species specific differences are reported in the literature. The sinking rates of growing diatoms are most likely more affected by growth conditions influencing buoyancy than by frustule silicification (Martin-Jézéquel et al., 2000). However, at the end of the exponential growth phase, when diatoms are not actively growing and are possibly already dead, an active buoyancy regulation is no longer possible. A stronger silicified frustule increases the dry weight of the cell and will therefore very likely also result in higher sinking rates. To evaluate the effect of iron fertilization on the export of carbon to the deep sea the effect on frustule silicification and thus weight might therefore be of greater importance. A stronger silicification of the frustules could also increase the cell wall strength and therefore provide a better grazing protection under unfavorable growth conditions. However, data presented in this manuscript and from other laboratory experiments indicate that frustule silicification does not increase in all diatom species under iron limitation (Takeda, 1998). The species specific differences in sinking rates reported by Waite and Nodder (2001) could therefore possibly also result from differences in frustule silicification besides species specific buoyancy regulation. *F. kerguelensis* is a bloom forming and very dominant species in the SO. If iron limitation would have such a strong effect on the energy budget of this species it is questionable if it would be so dominant in this region.

We therefore especially believe that the decreased sinking rates of *F. kerguelensis* cells after iron fertilization observed by Waite and Nodder (2001) result from decreased cellular silicification as shown in this study. However, it is important to mention in this context, that not all cellular Si is in the frustule, but that dissolved, internal Si pools can provide a significant amount (Martin-Jézéquel et al., 2000). These pools are usually low since Si is only taken up directly before cell wall formation and not stored over a longer time in most diatom species (Martin-Jézéquel et al., 2000). We only found significant differences in the BSi:POC, BSi:PON, and BSi:POP ratios between the high and low iron treatments of *F. kerguelensis* at day 34 (see Fig. 5) but not earlier during in the growth period at day 20, when higher division rates were observed and thus higher internal Si pools could be expected. We are therefore confident that the cellular Si concentrations taken from day 34, which are presented in this manuscript, mainly represent frustule bound Si and that our estimations concerning export are right. In *C. dictyota* the differences in the BSi:POC, BSi:PON, and BSi:POP ratios between the high and low iron treatments were still significant at day 43, when all treatments had reached highest chlorophyll concentrations. However, in this species the differences were even higher at day 31, when cells were still actively growing, at least in the low iron treatment (compare Fig. 4). Here, internal Si pools might play an important role.

The sediments of the SO mainly consist of diatom frustules of *Fragilariopsis kerguelensis* and this heavily silicified species has shown to increase silicification under low iron conditions in laboratory experiments including this study. Therefore, high accumulation rates of silica frustules in the sediment are probably mainly caused by strong silicification in this species under iron limitation. We therefore suggest that a decrease in silicification and lower sinking rates of *F. kerguelensis*, as observed by Waite and Nodder (2001), could decrease the dominant contribution of this species to the Si export under long-term iron fertilization in the SO. Less silicified frustules could additionally be more affected by grazing and remineralization and thus decrease carbon export as well. However, the observation that the cellular Si content of *C. dictyota* was not dependent on the iron availability, as well as the observation that *Trichotoxon* sp. and *Thalassiothrix* sp. did not change their sinking rates (Waite and Nodder, 2001), imply that other, also important SO diatom species, would not be affected. If all of those species would increase their cellular C concentrations similar to our observations for *C. dictyota*, this could even increase the carbon export with iron fertilization. It seems that the overall budget of carbon export after iron fertilization is mainly influenced by changes in the phytoplankton community structure (see Abellmann et al. (2006) and results presented in this manuscript).

In conclusion we suggest that changes in the implication of these changing elemental ratios for the cellular elemental composition of diatoms should be examined with

caution. Additionally, changes in the BSi:POC, BSi:PON, and BSi:POP ratios with changing iron availability should be carefully interpreted in terms of nutrient export. Changes in the sinking rates and grazing protection of dominant species might be of greater importance for biomass export and remineralization.

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